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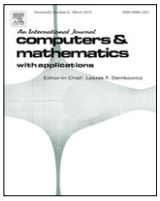

Theoretical and computational studies of some bioreactor models

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ABSTRACT

We study certain classical basic models for bioreactor simulation in case of batch mode with decay. It is shown that in many cases the two-dimensional differential system describing the dynamics of the substrate and biomass concentrations can be reduced to an algebraic equation for the biomass together with a single differential equation for the substrate. Then from an analogy with the Henri–Michaelis–Menten enzyme kinetic mechanism a simple model is proposed for a bioreactor in batch mode with decay. Two more models are also proposed taking into account the phases of microbial growth. Some properties of these two models are studied and compared to classical Monod type models using computer simulations.

1. Introduction

It has been often mentioned in the literature that Monod type microbial growth models describe adequately bio-processes appearing in bioreactors, under certain favorable conditions when micro-organisms actively produce specific enzymes for the degradation and consumption of nutrient substrates and grow at the maximum possible rate [1,2]. However, conditions in bio-reactors sometimes become unfavorable, microbial growth may be inhibited and bio-technological processes may go out of control. In this work we study theoretically and computationally several Monod type models, taking into account phases in microbial growth under unfavorable conditions. To this end several familiar bioreactor models are theoretically analyzed with respect to properties of their solutions. In particular we investigate (in the spirit of [3]) some models of batch mode bioreactors with decay of the form:

$$\begin{aligned} s_t' &= -\alpha\mu(s)x, \\ x_t' &= \mu(s)x - k_d x, \end{aligned} \tag{1}$$

with positive initial conditions and positive α and k_d. The function $\mu(s) \geq 0$ in (1) is defined for $s \geq 0$ and may take various forms, see e.g. [1]. The emphasis is on the case when $\mu(s)$ is the Monod function [4], the Webb function [5] and its particular cases: the Haldane function [6] and the Andrews function [7]. In this paper it is shown that if $\int ds/\mu(s)$ exists then model (1) can be simplified to a single differential equation plus an algebraic relation of the form $x = x(s)$. As examples the corresponding algebraic functions are derived for each of the four above functions.

In the last part of this work we consider a class of models for bacterial growth proposed in [8] as an alternative to (1). Three particular models from this class, inspired from the Henri–Michaelis–Menten enzyme kinetic mechanism, are studied computationally. It is shown that these type of models are an alternative to Monod type models for bioreactors with decay and that they lead to similar solutions. The figures illustrating the theoretical results of this paper have been obtained using the ordinary differential equations solver LSODE from ODEPACK [9].

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2. The classical simple bioreactor model

A simple continuous bioreactor involving a single nutrient substrate s and one bacteria strain x is often described by the Monod type differential system:

$$\begin{aligned} s_t' &= -\alpha\mu(s)x - q(s - s_{in}), \\ x_t' &= \mu(s)x - qx, \end{aligned} \tag{2}$$

with initial conditions s_0, x_0 in $t = 0$; t is the time, $s(t)$ and $x(t)$ are the concentrations at time t of the substrate and of the biomass of bacteria; α, q, s_{in} are nonnegative constants. More precisely $1/\alpha$ is the growth yield, s_{in} is the concentration (g/l) of fresh nutrient and $q = F/V$, F being the inflow (liters/hour) and V being the volume (in liters) of the chemostat. In case of batch mode there is no inflow and $q = 0$.

The function $\mu(s)$ represents the specific growth rate of the biomass. More than 30 functions $\mu(s)$ have been discussed in [1]. They can be classified as follows:

1. Models representing only the bacterial growth (e.g. Monod), most are rational functions in s;
2. Models representing bacterial growth including the effect of substrate inhibition (e.g. Haldane, Andrews), most are rational functions in s;
3. Models for bacterial growth including the effect of product inhibition, most are also rational functions in s and x;
4. Models describing the influence of the pH value or other experimental conditions on bacterial growth.

The following functions are often used in the literature:

(i) Monod function [4]:

$$\mu_m(s) = \mu^* \frac{s}{K + s}; \tag{3}$$

(ii) Webb function [5]

$$\mu_w(s) = \mu^* \frac{s(1 + \beta s/K_i)}{K + s + s^2/K_i}. \tag{4}$$

(iii) Haldane function [6]:

$$\mu_h(s) = \mu^* \frac{s}{(K + s)(1 + s/K_i)}; \tag{5}$$

(iv) Andrews function [7]:

$$\mu_a(s) = \mu^* \frac{s}{K + s + s^2/K_i}. \tag{6}$$

We note that all parameters μ^*, K and K_i in formulae (3)–(6) are positive and represent different physical/biological quantities, so strictly speaking we should have used a different notation for all the parameters. Haldane and Andrews functions are equivalent in the sense that using a transformation of the parameters, function (5) can be written in the form (6):

$$\mu_h(s) = \mu^* K_i/(K + K_i) \frac{s}{KK_i/(K + K_i) + s + s^2/(K + K_i)};$$

and vice versa. Functions (4)–(6) are used to model enzyme inhibition at high substrate concentrations [1]. Andrews function is a special case of the Webb function for $\beta = 0$. The Monod, Webb, Haldane and Andrews functions are visualized in Fig. 1 for the following values of the parameters: $\mu^* = 3$, $K = 2.3$, $K_i = 1$, $\beta = 0.1$.

In the literature μ^* is sometimes denoted μ_{max}, meaning that μ^* is maximum of the function μ. Indeed, $\mu^* = \mu_{max}$ is true in the case of the Monod function as μ_m is monotone increasing and μ^* is an asymptotic limit for $\mu_m(s)$. Webb, Haldane and Andrews functions are unimodal, and the parameter μ^* does not represent the maximum of $\mu_w(s)$, resp. $\mu_h(s)$, $\mu_a(s)$. Haldane and Andrews functions achieve their maxima at $s^* = \sqrt{KK_i}$ and both tend to zero with $s \longrightarrow \infty$. The expressions for the maxima of the functions (5), (6) are as follows: $\max \mu_h(s) = \mu_h(s^*) = \mu^*/(1 + \sqrt{K/K_i})^2$; $\max \mu_a(s) = \mu_a(s^*) = \mu^*/(1 + 2\sqrt{K/K_i})$. We also note that function (4) tends to $\beta\mu^*$ when $s \longrightarrow \infty$.

3. Classical bioreactor model solution in batch mode

Let us focus on the solutions of the bioreactor model (2) in case of batch mode and $\mu(s)$ being one of the functions (3)–(6). The batch mode can be seen as a special case of (2) with $q = 0$:

$$\begin{aligned} s_t' &= -\alpha\mu(s)x, \\ x_t' &= \mu(s)x. \end{aligned} \tag{7}$$

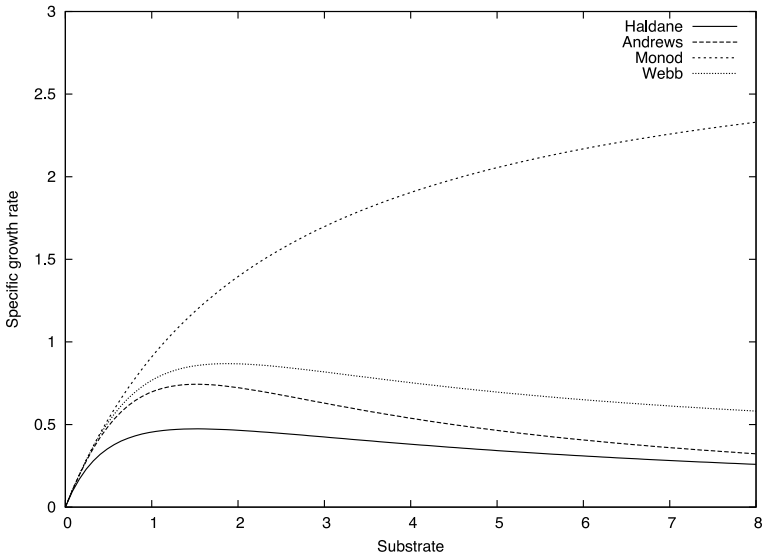

Fig. 1. Monod, Webb, Haldane and Andrews functions with values of the parameters: $\mu^* = 3,\ K = 2.3,\ K_i = 1,\ \beta = 0.1$.

It is known that in model (7) x can be solved in terms of s and thus the two-dimensional differential system can be replaced by a single differential equation. Moreover it can be seen that for any nonnegative $\mu(s)$, function $s(t)$ is monotone decreasing and function $x(t)$ is monotone increasing. This is an evident consequence of (7) together with the fact that by their nature, α and $x(t)$ are positive. In the literature it has been often pointed out that the form of function $\mu(s)$ may play a decisive role for the inhibition of bacterial growth. Two extremal cases should be considered: prolonged depletion and prolonged excess of s. Concerning the first case, we note that no nonnegative function $\mu(s)$ can cause decay of the biomass even long after the substrate has been totally exhausted. If we need to model the death of bacteria due to prolonged depletion of nutrient substrate then we need to include a decay term in the second equation of system (7). This fact is also well-known, see [1]. Concerning inhibition due to (toxic) excess of substrate, functions (4)–(6) prove to be useful. Note that the Monod function (3) cannot contribute to the inhibition of substrate uptake due to excess of s, whereas functions (4)–(6) do cause inhibition.

One of the main purposes of this work is to show that: (i) in many cases the two-dimensional differential system (1) can be replaced by a single differential equation plus an algebraic relation; (ii) the inhibition effects that are usually aimed by means of function $\mu(s)$, can be achieved by other means.

3.1. Batch bioreactor with decay

Because of the above reasons, in what follows the studied model will be the classical model (1) for a bioreactor with decay term $-k_d x$.

Proposition 1. *In the two-dimensional system (1), if $1/\mu(s)$ admits an antiderivative function, then x can be solved in terms of s and the system can be reduced to a single differential equation in s.*

Proof. Following an idea of Volterra, see for example [10], the ratio of the second equation to the first equation of system (1) leads to:

$$\frac{dx}{ds} = \frac{1}{\alpha}\left(\frac{k_d}{\mu(s)} - 1\right). \tag{8}$$

Let ϕ be an antiderivative of $1/\mu$: $\phi(s) = \int ds/\mu(s)$, then, taking into account the initial conditions $s(0) = s_0, x(0) = x_0$:

$$x(s) = \frac{1}{\alpha}[k_d(\phi(s) - \phi(s_0)) - (s - s_0)] + x_0, \tag{9}$$

which proves the proposition. □

Let us now apply Proposition 1 to the above mentioned functions $\mu(s)$.

Proposition 2. *In the case of Monod function, the second equation in system (1) can be expressed as:*

$$x(s) = x_0 + \frac{1}{\alpha\mu^*}[k_d K \ln(s/s_0) - (\mu^* - k_d)(s - s_0)]. \tag{10}$$

The proof follows from a simple calculation of $\phi(s) = \int ds/\mu(s)$, $\mu(s)$ being the Monod function and replacing it in (9). □

Similar calculations can be also done with the Webb function and its particular cases of Andrews and Haldane functions leading to:

Proposition 3. *In the case of the Webb function we have*

$$\phi(s) = \frac{1}{\mu^*}\left[(\ln(as+1))/a + K\ln(s/(as+1)) + \frac{1}{K_i}(s/a - (\ln(as+1))/a^2)\right]$$

with $a = \beta/K_i$ so that the second equation of system (1) can be expressed as:

$$x(s) = x_0 + \frac{1}{\alpha\mu^*}\left[\left(\frac{k_d}{a} - \frac{k_d}{K_i a^2}\right)\ln\left(\frac{as+1}{as_0+1}\right) + k_d K \ln\left(\frac{s(as_0+1)}{s_0(as+1)}\right) + \left(\frac{k_d}{K_i a} - \mu^*\right)(s-s_0)\right]. \tag{11}$$

Proposition 4. *In the case of Haldane and Andrews functions, the second equation of system (1) respectively reduces to:*

$$\begin{aligned} x(s) &= x_0 + \frac{1}{\alpha\mu^*}\left[k_d K \ln\frac{s}{s_0} + \left(k_d + \frac{k_d K}{K_i} - \mu^*\right)(s-s_0) + \frac{k_d}{2K_i}(s^2 - s_0^2)\right]. \\ x(s) &= x_0 + \frac{1}{\alpha\mu^*}\left[k_d K \ln\frac{s}{s_0} + (k_d - \mu^*)(s-s_0) + \frac{k_d}{2K_i}(s^2 - s_0^2)\right]. \end{aligned} \tag{12}$$

Formulae (12) can be obtained directly from (9) by calculating $\phi(s)$ with the Haldane and Andrews formulations, or directly from (11) with β tending to zero for Andrews function.

Similar calculations can be done each time $\int ds/\mu(s)$ exists. This has been illustrated by the above examples. Thus it has been demonstrated that, even in the case of a model with decay, the two-dimensional differential system can be often reduced to a single differential equation for the substrate together with an algebraic equation for the biomass. Let us see next that in the case of no decay ($k_d = 0$) the differential equation can sometimes be solved in terms of t as a function of s.

3.2. Special case of a model without decay

In the special case when $k_d = 0$ it is known that the model with the Monod function can be solved in terms of two functions $s = s(t)$ and $x = x(t)$, more precisely, when the system is close to the steady state the differential equation has an approximate explicit solution using the Lambert W-function:

$$s(t) = KW\left(\frac{s_0}{K}\exp\left(\frac{s_0 - C\mu^* t}{K}\right)\right)$$

with $C = \alpha x_0 + s_0$. $W(x)$ is the Lambert function which is the inverse to the function $y = xe^x$, i.e. such that $x = W(x)\exp(W(x))$ [11,12]. This solution can be obtained in the special case of the steady state with the Monod function.

Let us see next that the above expression (10) and (12) for $x(s)$ lead to differential equations that can be solved in terms of time as a function of s, that is $t = t(s)$.

When $k_d = 0$ for any model, Eq. (8) reduces to $dx/ds = -1/\alpha$ and leads to

$$x(s) = x_0 - \frac{1}{\alpha}(s - s_0).$$

The latter expression, when respectively replaced in the expression of $s'(t)$ in (7) leads to a differential equation with separable variables which can be easily solved for the Monod, Andrews and Haldane functions. Recall that $\int ds/(s(C-s)) = \frac{1}{C}ln(s/(s-C))$ for any constant C such that $C - s > 0$. The solutions are:

- For the Monod function:

$$t = \left[\frac{K}{C}\ln\left(\frac{s_0(C-s)}{s(C-s_0)}\right) + \ln\left(\frac{C-s}{C-s_0}\right)\right]\Big/\mu^*.$$

- For the Andrews function:

$$t = \left[\frac{K}{C}\ln\left(\frac{s_0(C-s)}{s(C-s_0)}\right) + \left(1 + \frac{C}{K_i}\right)\ln\left(\frac{C-s}{C-s_0}\right) + \frac{s-s_0}{K_i}\right]\Big/\mu^*.$$

- For the Haldane function:

$$t = \left[\frac{K}{C}\ln\left(\frac{s_0(C-s)}{s(C-s_0)}\right) + \left(1 + \frac{C+K}{K_i}\right)\ln\left(\frac{C-s}{C-s_0}\right) + \frac{s-s_0}{K_i}\right]\Big/\mu^*.$$

with $C = \alpha x_0 + s_0$.

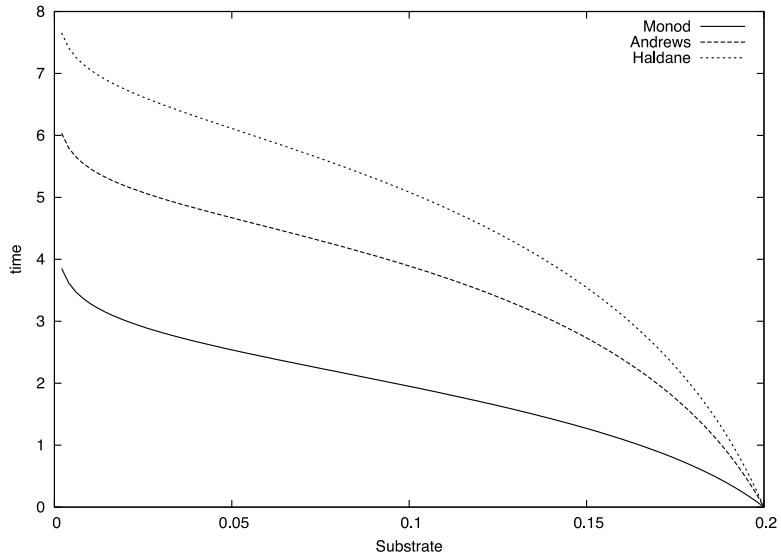

Fig. 2. Time as a function of substrate for the Monod, Haldane and Andrews functions.

Remark. Analogous calculations can be done for any function $\mu(s)$ leading to $s'(t)$ in (7) being a differential equation with separable variables once x is replaced by its expression $x(s)$.

Fig. 2 presents the above functions $t = t(s)$ obtained for the following values of the parameters: $K = 0.1,\ K_i = 0.1,\ \alpha = 1./23,\ \mu^* = 1.35,\ s_0 = 2.0K,\ x_0 = 0.25K/\alpha,\ c = s_0 + \alpha x_0$. The computed solutions are dimensionless. Note that the curves of Fig. 2 should be read from right to left.

It can be checked that the three curves $t(s)$ are symmetric to the corresponding $s(t)$ with respect to the first diagonal, that is the line $t = s$. Thus when t tends to ∞, $s(t)$ tends to 0 and symmetrically when s tends to 0, then t tends to $+\infty$. Hence the three solutions $s(t)$ are asymptotic to the vertical axis when s tends to 0.

These equations express the time t as a function of the substrate s which is not very convenient. But, for any t, they can be solved using a classical solver such as Raphson–Newton and hence they can be written as functions $s = s(t)$. This is useful when one needs to compute the value of the substrate at a given time t without computing all the preceding values with some step.

Let us see next that a model for a batch bioreactor with decay can be obtained using a different approach.

4. A model of batch bioreactor with decay suggested by enzyme kinetics

4.1. Motivation

The following considerations come from the analogy between the reactions in a bioreactor and enzyme kinetics. Let us consider the well-known Henri–Michaelis–Menten (HMM) model in enzyme kinetics:

$$\begin{aligned} ds/dt &= -k_1 es + k_{-1} c, \\ de/dt &= -k_1 es + (k_{-1} + k_2) c, \\ dc/dt &= k_1 es - (k_{-1} + k_2) c, \end{aligned} \tag{13}$$

with initial conditions $s(0) = s_0,\ e(0) = e_0,\ c(0) = 0$. In system (13) s, e and c are respectively the concentrations of the substrate, the enzyme and the complex (bounded enzyme).

Remark. We have omitted the differential equation for the product as being decoupled. Besides the form of the equation for the product depends on the mechanism of the biochemical process, see e.g. [13].

Using that $de/dt + dc/dt = 0$ implies $e(t) + c(t) = e_0 = \text{const}$ and thus $c = e_0 - e$ (or $e = e_0 - c$), system (13) reduces to two equations either for the variables s, e:

$$\begin{aligned} ds/dt &= -k_1 es + k_{-1}(e_0 - e), \\ de/dt &= -k_1 es + (k_{-1} + k_2)(e_0 - e), \\ s(0) &= s_0, \qquad e(0) = e_0; \end{aligned} \tag{14}$$

or for the variables s, c:

$$\begin{aligned} ds/dt &= -k_1(e_0 - c)s + k_{-1}c, \\ dc/dt &= k_1(e_0 - c)s - (k_{-1} + k_2)c, \\ s(0) &= s_0, \qquad c(0) = 0. \end{aligned} \tag{15}$$

We shall next look for comparisons and analogies with the substrate microbial dynamics in a simple bioreactor with one limiting substrate and one microbial strain.

Recall that the Michaelis–Menten dynamics of substrate uptake:

$$ds/dt = -Vs/(K_m + s), \quad V = k_2 e_0 = \text{const}, \tag{16}$$

is obtained from Eq. (15) by an approximation based on the two (related) assumptions (i) $s_0 \gg e_0$ and (ii) $c' = \text{const}$.

We see from (16) that the Michaelis–Menten rate of substrate uptake is a rational function of s involving a denominator $K_m + s$. It is this denominator which slows down the rate of substrate uptake from exponential one (if the denominator is absent) up to a hyperbolic one (or even almost uniform for large values of s). Three important points should be recalled: (i) the MM-uptake (16) is an approximation of the correct substrate uptake given by any one of the systems (13)–(15); (ii) any one of the systems (13)–(15) models the substrate uptake correctly (as based on mass action law); (iii) none of systems (13)–(15) makes use of any rational expressions involving variables in denominators. In addition let us recall that, the assumption $s_0 \gg e_0$ may be far from reality, when modeling substrate microbial dynamics of a simple bioreactor, see e.g. [14,15].

We shall next propose two alternative approaches inspired from the correct enzyme kinetic descriptions (13)–(15). The first approach consists in hybridizing systems (14), (15) into a new (but similar) system of two ODE's taking into account certain analogies between biomass and enzymes. In this case the model shall involve a single microbial variable, say x, whose dynamics possesses certain features of the dynamics of the enzyme variables (free or bounded).

The second approach is to start from the original correct dynamical system (13). In this case we shall look for analogies between the enzyme kinetic variables e, c and two variables, say x, y, corresponding to different microbial phases.

We note that both approaches ensure that the dynamics of the substrate uptake is similar to Monod dynamics (at least for $s_0 \gg e_0$). Thereby this is achieved without introducing a rational function of the substrate variable s in the right-hand-side. In addition, the second approach offers a better description of microbial dynamics due to the employment of two microbial phases. The advantages of using microbial phases have been advocated by several authors, see e.g. [16–18,2].

We start with the first approach which is more simple. Our aim is to construct a model in analogy to models (14), (15) extracting from the latter appropriate information for modeling microbial growth. Passing from enzyme kinetics to microbial dynamics it seems reasonable to assume that the rate constant k_{-1} corresponding to the reverse enzyme reaction is zero. (Assuming $k_{-1} \neq 0$ in a bacterial growth model may mean that under certain conditions bacteria throw up (vomit) some of the already swallowed nutrient substrate.) This simplifies systems (14), (15) to:

$$\begin{aligned} ds/dt &= -k_1 es, \\ de/dt &= -k_1 es + k_2(e_0 - e), \end{aligned}$$

and, resp. (for s and c):

$$\begin{aligned} ds/dt &= -k_1 es = -k_1(e_0 - c)s, \\ dc/dt &= k_1 es - k_2 c = k_1(e_0 - c)s - k_2 c. \end{aligned}$$

Remark. Note that systems (14), (15) (and the two above systems) are direct consequences of the assumption $e + c = \text{const}$. The latter is a realistic restriction reflecting a structural property of the model. This restriction remains in large extent valid while modeling bacterial growth (although, if necessary, can be slightly modified). On the contrary the assumption $c' = 0$ used in the derivation of (16) is quite unrealistic in modeling microbial growth.

4.2. A simple model

The similarity of the curves obtained with models (14), (15) and those obtained with models (1) with any of the three μ-functions (3)–(6) suggests that microbial growth can be modeled with some modification/hybridization of systems (14), (15). Thereby there will be no rational functions of the substrate variable in the formulation of the model, which simplifies the latter. The idea arises from the similar roles played by enzymes and microorganisms in relation to substrate uptake/consumption.

The following Model 1 illustrates this idea. The model is a hybrid of the two systems (14) and (15). We assume that bacteria behave similar to free enzymes w.r.t. substrate uptake/consumption. Denoting bacteria biomass by x we may expect that equation $ds/dt = -k_1 xs$ models quite adequately the consumption of nutrient substrate by bacteria. (A more sophisticated model accounting for the inhibition on the uptake for excessive values of s may involve an additional term such as $+k_i s$.) On the other side bacteria behave similarly to bounded enzymes when it comes to bacterial dynamics (growth/decay). The above reflections lead us to the following simple model.

Model 1. A simple substrate microbial dynamics is described by means of the following system of two ODE's:

$$\begin{aligned} ds/dt &= -k_1 xs, \\ dx/dt &= \bar{k}_1 xs - k_3 x, \end{aligned} \tag{17}$$

with initial conditions $s(0) = s_0,\ x(0) = x_0$.

Remark. A modeler of microbial-nutrient system should not feel restricted to assign precisely one specified form of the enzyme (free or bounded) to a specific microbial state. At this point the modeler should feel free to use any one of these two systems as a base for his microbial grows model and to use elements of the other system. Indeed, it seems to us possible that when being in a specific state, bacteria may behave sometimes as a free, and sometimes as a bounded enzyme. For example while in active state, bacteria consume nutrients like free enzymes uptake substrate, but these bacteria decay like bounded enzymes pass to free ones. Thus, in the right-hand side of the second equation of Model 1 : $dx/dt = \bar{k}_1 xs - k_3 x$, the first term corresponds to the term $k_1 es$ from (15), whereas the second term $-k_3 x$ corresponds to the term $-k_2 c$ from (15).

We wish to also remark that Model 1 is intentionally simplified in order to demonstrate our ideas. Namely, to replace the rational function μ by means of polynomial function in the right-hand sides, and to show that this simplified model does (almost) the same work as model (1) with Monod function. Note that if we substitute $\mu(s) = s$ in (1) then we obtain the model:

$$\begin{aligned} ds/dt &= -\alpha xs, \\ dx/dt &= \alpha xs - k_3 x, \end{aligned}$$

which is a special case of (17). Model (17) does not model realistically bacterial growth well for large values of s, but many sources report that the Monod kinetics model (1) does not work well for large s too.

As in the case of the batch bioreactor model of the preceding section, we have the next proposition.

Proposition 5. *System* (17) *reduces to a single differential equation.*

Proof. As has been done in the preceding section using a Volterra idea the ratio of the second equation to the first equation of system (17) leads to:

$$dx/ds = -\bar{k}_1/k_1 + k_3/(sk_1)$$

which can be integrated as:

$$x = -\frac{\bar{k}_1}{k_1} s + \frac{k_3}{k_1} \ln(s) + M.$$

The constant M is obtained from the initial conditions x_0 and s_0.

Hence the single differential equation with algebraic relation:

$$\begin{aligned} s' &= -k_1 xs, \\ x &= x_0 + \frac{k_3}{k_1} \ln \frac{s}{s_0} - \frac{\bar{k}_1}{k1}(s - s_0) \end{aligned} \tag{18}$$

is equivalent to (17). □

It can be remarked that the expression for x in (18) is very close to the one for x in (10).

Let us now see that if the coefficients for the Monod system (10) are known it is possible to obtain some conditions on the coefficients $k_1, \bar{k}_1, k_3$ of (17) or equivalently of (18) so that the two models give close solutions. A simple condition is that the maximum in the solution for x in (10) is identical to the one for x in (18). Let us call s_m (resp. s_{m1}) the values of s for which $x(s)$ has its maximum, then: $x'(s) = 0$ in (10) leads to $s_m = k_d K/(\mu^* - k_d)$ and $x'(s) = 0$ in (18) leads to $s_{m1} = k_3/\bar{k}_1$.

The identification of s_m and s_{m1} leads to a ratio $k_3/\bar{k}_1$ which depends only on the coefficients of the Monod model (10).

Example 1. As an example model 1 is tested on real data for the growth of the bacterial strain *Escherichia Coli* on glucose. The solutions obtained with the two systems (10) and (18) are plotted in Fig. 3. The *E. Coli*–glucose system is modeled by means of (10) using the following values for the coefficients, see [3]: $k_d = 0.25,\ K = 0.004,\ \alpha = 1/23$. The initial conditions are $s_0 = 2\,K,\ x_0 = 0.25\,K/\alpha$, and the plotted solutions are dimensionless. The corresponding coefficients for system (17) or equivalently for (18) are $k_1 = 0.67,\ k_3 = 0.125, \bar{k}_1 = 0.551$.

Fig. 3 shows that the solutions computed with the classical model with the Monod function decrease slightly faster than those computed with model (17). In the following section it will be shown that a better agreement can be obtained by means of more sophisticated models.

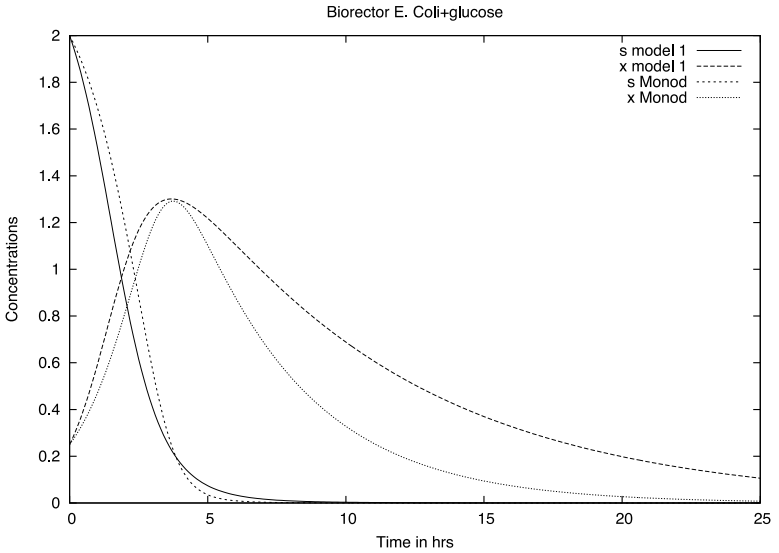

Fig. 3. *E. Coli*–glucose system modeled by systems (10) and (18).

5. Models using microbial growth phases

Bacterial growth in batch culture involves several phases such as: lag, log, stationary and death phases, cf. e.g. [1]. The first three phases are modeled by the logistic curve which solves the Verhulst–Pearl ODE: $dx/dt = ax(1 - x)$. However, Verhulst–Pearl model does not involve variables corresponding to bacteria biomass in specific phases, neither does it involve the dynamics of the nutrient substrate.

In the literature on bioreactors/chemostats one can rarely find models involving microbial growth phases. Some examples of such "structured" models can be found in [16–18,2]. The models proposed and studied in these papers are not inspired by models from enzyme kinetic; a characteristic feature of these models is that they employ Monod type specific growth functions that are rational functions of the substrate variable. It is important to note that models using phases are reported to be biologically adequate in many realistic situations and therefore deserve special attention.

5.1. Introducing phases in microbial growth models

Aiming at possibly simpler models we subdivide the microbial population into two subgroups:

(i) microorganisms in the lag and stationary phases are classified into one subclass with biomass denoted x. It is assumed that microorganisms in that class experience unfavorable growth conditions and are not able to immediately produce enzymes;

(ii) active (viable) microorganisms in log phase, denoted y, possessing a complete set of active enzymes.

Bacteria in the dying state shall be modeled by decay terms and need not be assigned to a special subgroup.

Under these assumptions there is a clear analogy between a microbial–nutrient system and an enzyme–substrate system. A possible start is to relate the sub-population x to free enzymes e and the sub-population y to bounded enzymes c and then make suitable realistic modifications. As in the preceding section, plausible dynamic models of a batch mode bioreactor can be constructed in analogy to the HMM-law of enzyme kinetic (13). Assuming $k_{-1} = 0$, system (13) looks as follows:

$$\begin{aligned} ds/dt &= -k_1 xs, \\ dx/dt &= -k_1 xs + k_2 y, \\ dy/dt &= k_1 xs - k_2 y, \end{aligned} \tag{19}$$

with initial conditions $s(0) = s_0,\ x(0) = x_0,\ y(0) = y_0$.

A structural property of this system is $x + y = x_0 = \text{const}$ but when modeling a bacterial–substrate system we can slightly deviate from this property.

5.2. Two microbial growth models using phases

Adding appropriate additional terms in (19) a variety of models can be obtained. Below we study numerically two models of a microbial–nutrient batch mode bioreactor proposed in [8], the second model has been slightly modified. Both models involve two microbial growth phases.

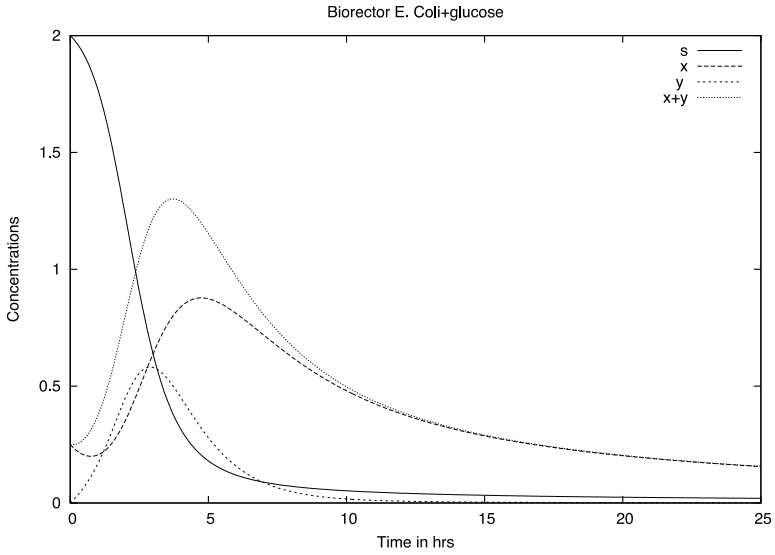

Fig. 4. Microbial growth, model (20).

Model 2. Consider the following system of ODE's:

$$\begin{aligned} ds/dt &= -k_1xs - \beta ys, \\ dx/dt &= -k_1xs + k_2y - k_dx^2, \\ dy/dt &= k_1xs - k_2y + \beta ys, \end{aligned} \tag{20}$$

with the initial conditions $s(0) = s_0,\ x(0) = x_0,\ y(0) = y_0$. One may see that system (20) is obtained from system (19) by adding some additional terms. The terms participating in system (20) have the following meaning:

k_1xs — models the consuming of s by bacteria x and the transition of (fasting) bacteria x into (viable, active) bacteria y;

βys — models the consuming of s by bacteria y and the increase of bacteria biomass y due to nutrition and reproduction;

k_2y — models the random transitions of bacteria from class y into class x;

k_dx^2 — models competition and decay of (starving) bacteria x.

The meaning of the coefficients k_1 and k_2 are similar to that in the enzymatic HMM system (13).

The presented numerical experiments concern as before the tandem microbial-substrate system described in [3]. The solutions are visualized on Fig. 4. The parameters in system (20) are chosen as follows: $k_1 = 0.23$; $k_2 = 0.85$; $k_d = 0.3$; $\beta = 1.0$; and the initial conditions are: $s_0 = 2.0$; $x_0 = 0.25$; $y_0 = 0$. A fast growth of the population of active bacteria is observed during a sufficient supply of substrate nutrient s. The decay in the total amount of biomass $x + y$ due to substrate depletion is also clearly seen in Fig. 4.

As can be seen on Fig. 5 model (20) provides solutions for s and the total biomass $x + y$ which are very close to the one obtained with the model (1) with Monod μ-function (3).

Model 3. Consider next the following system of ODE's:

$$\begin{aligned} ds/dt &= -k_1xs - (\alpha + \beta)ys, \\ dx/dt &= -k_1xs + k_2y + \alpha ys - k_xx, \\ dy/dt &= k_1xs - k_2y + \beta ys - k_yy, \end{aligned} \tag{21}$$

with the initial conditions $s(0) = s_0,\ x(0) = x_0,\ y(0) = y_0 = 0$.

The new terms in system (21) have the following meaning:

k_xx — models the decay of bacteria x;

k_yy — models the decay of bacteria y.

The meaning of the remaining coefficients/terms is similar to that in system (20) with one difference: in (21) the term αys with $\alpha \leq \beta$ models a part of (overfed, poisoned) bacteria y which pass from compartment y to compartment x.

In Fig. 6 the solutions of model (21) are visualized and the comparison of the solutions obtained with model (1) with Monod μ-function (3) and model 3 is reported in Fig. 7. The values of the coefficients are $k_1 = 0.05$, $k_2 = 0.85$, $k_x = k_y = 0.23$, $\beta = 1.5$ and $\alpha = 1.35$.

The numerical simulations show that all three models (17), (20) and (21) adequately reflect the microbial growth limitation by nutrient depletion. The presence of more parameters and terms allows us the freedom to tune the model better to particular realistic situations. The behavior of the computed solutions is close to the experimentally observed behavior of microbial growth under both favorable and unfavorable environments.

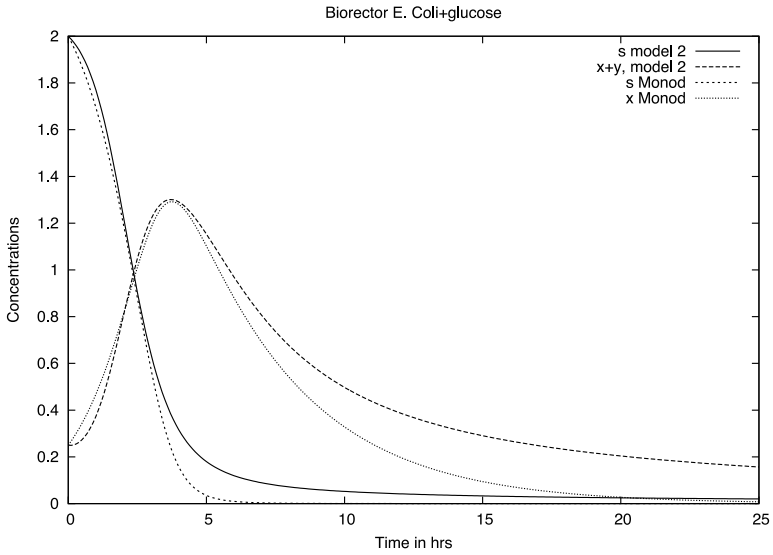

Fig. 5. Microbial growth, comparing models (1) and (20).

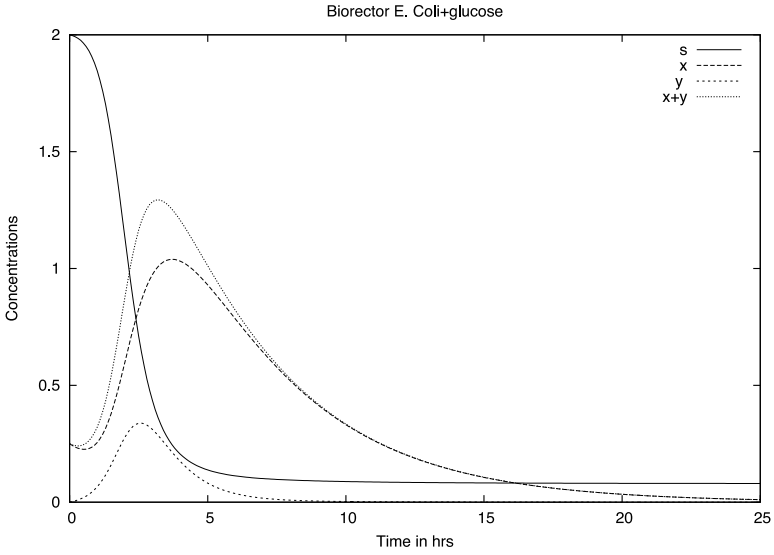

Fig. 6. Microbial growth model (21).

It has been experimentally shown that in the case of a batch bioreactor with decay the coefficients of the proposed models can be adapted in a way that the computed solutions with these models are close to the one obtained with the Monod model. In fact the main condition is that the maxima of the solutions for the total biomass are the same and obtained at identical values of time t with each model. Once this condition has been stated the coefficients of the HMM like model such as models 1–3 can be obtained with an optimization program allowing the computation of the minimum distance between the maximum of the Monod solution and the one of new model solution.

6. Conclusion

In this paper some theoretical properties of the Monod type models for a bioreactor with decay have been investigated. It has been shown that in most cases the two-dimensional differential system of the model can be reduced to a one-dimensional one together with an algebraic equation. The particular cases of the Monod, Haldane and Andrews functions have been detailed and the resp. one-dimensional systems have been discussed. It has been proved that when there is no decay an explicit solution can be obtained in terms of the time as a function of the nutrient. The reciprocal function can then be numerically obtained using some function solver. Some models inspired by adaptations from enzyme kinetics

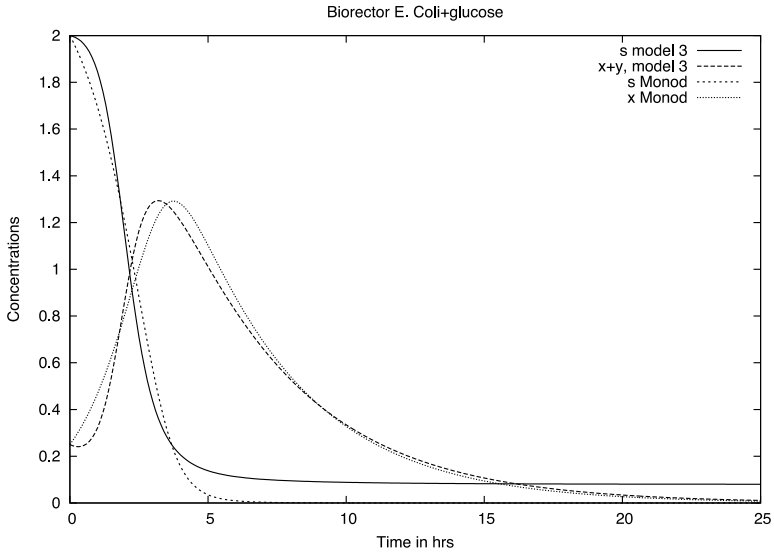

Fig. 7. Microbial growth models (1) and (21).

mechanisms have been proposed and their solutions have been compared to the ones provided by Monod type models. It has been shown that with appropriately chosen coefficients, these models are good alternatives to classical Monod type models, especially when introducing microbial phases. Some hints to compute the parameters of the proposed models have been given. The presented computational experiments concern the growth of the microbial strain *E. Coli* on glucose substrate. Computational studies related to experimental data on microbial growth reported in [19,20] are in progress. Future work will concern a more complete theory of the relations between Monod type models and Henri–Michaelis–Menten type models adapted to bioreactors.

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